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Emergence of Disseminated Infections Due to *Geosmithia argillacea* in Patients with Chronic Granulomatous Disease Receiving Long-Term Azole Antifungal Prophylaxis▽

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We report two cases of invasive infections due to *Geosmithia argillacea*, an emerging mold, in patients with chronic granulomatous disease receiving prolonged azole antifungal prophylaxis. One patient died despite receiving a combination of four antifungals, and the other developed cerebral and medullary lesions under a combination of caspofungin, posaconazole, terbinafine, and gamma interferon.

CASE REPORTS

Patient 1. A 14-year-old girl with chronic granulomatous disease (CGD; autosomal recessive form implying the CYBA gene encoding subunit $p22^{phox}$) was admitted to Nancy University Hospital (Nancy-F) for allogeneic peripheral blood stem cell transplantation using a matched unrelated donor. Her medical history included a prior undocumented granulomatous pulmonary mass requiring lobectomy in 2006. She received a primary antifungal prophylaxis, with 200 mg voriconazole twice a day (b.i.d.) from the day of grafting. On day 43 postgraft, she developed acute grade II graft-versus-host disease, requiring methylprednisolone. On day 60, galactomannan was detected in serum, and a computed tomography (CT) scan revealed a T1 spondylodiscitis with a paravertebral abscess. Liposomal amphotericin B (3 mg/kg of body weight per day), combined with caspofungin (70-mg loading dose, followed by 50 mg daily), was initiated. A biopsy of the paravertebral abscess was performed. Microscopic examination of histological sections stained with periodic acid-Schiff stain, hematoxylin-eosin, and Gomori methenamine silver revealed branching, septate hyphae, and many vesicular swellings of different sizes (Fig. 1A). Cultures of the biopsy specimen (isolate 1) rapidly grew brownish colonies on Sabouraud agar at 30°C. The microscopic morphology, with penicillate conidiophores attached to hyaline septate hyphae, resembled that of a *Penicillium* species. On day 143, she had a persistent cough with dyspnea and fever and received empirical antibiotics. On day 198 postgraft, she experienced a seizure, and cerebral magnetic resonance imaging (MRI) revealed disseminated cerebellar and cerebral abscesses. A cerebral biopsy was not performed. She subsequently received terbinafine (250 mg b.i.d.) and then flucytosine (6 g/day) in addition to liposomal amphotericin B and caspofungin but died on day 258 postgraft.

Patient 2. A 30-year-old patient with X-linked recessive CGD was admitted in March 2010 at the Centre d'Infectiologie Necker-Pasteur (Paris-F) with bilateral pulmonary infiltrates ($\geq$4 cm) and a thoracic subcutaneous abscess with local rib lysis.

He previously experienced relapsing otitis media and *Mycobacterium bovis* adenitis. He also had developed 3 prior episodes of invasive aspergillosis, the latest being in May 2009 and related to *Aspergillus nidulans*, for which he received voriconazole. Subsequently, he was transiently colonized by *Penicillium chermesinum* and *Scopulariopsis brevicaulis* (both confirmed by internal transcribed spacer [ITS] sequencing). Bronchoalveolar lavage fluid culture and biopsy of the subcutaneous lesion revealed septate hyphae upon direct examination and a filamentous fungus thought to be *Paecilomyces variotii* in culture (isolate 2). After the definitive fungal identification (see below), triple antifungal therapy consisting of posaconazole, caspofungin (150 mg/day), terbinafine, and gamma interferon was initiated. In October 2010, the patient had gained weight

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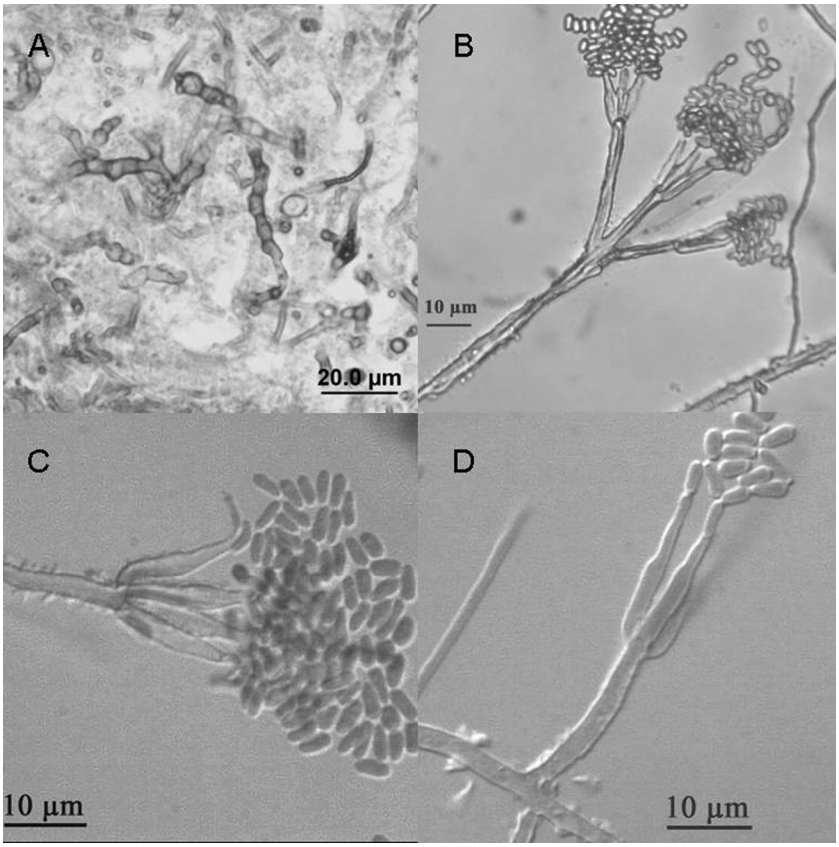

FIG. 1. (A) Hematoxylin-eosin (H&E) staining of the paravertebral abscess biopsy specimen showing multiple septate hyphae with vesicles. (B to D) Morphology of conidiophores, conidiogenous cells, and conidia of *Geosmithia argillacea*.

and returned to work, and he receives at present all the above-mentioned drugs, with a marked improvement of both pulmonary and subcutaneous lesions. However, in December 2010, the patient developed multiple aseptic brain and then medullary abscesses while receiving the above-described antifungal strategy.

Identification of the isolates was carried out at the Institut Pasteur, Paris-F, by combining morphological characteristics and sequences of the ITS1-5.8S-ITS2 region of ribosomal DNA (rDNA). Cultures on 2% malt extract agar at 30°C were low, with a velutinous texture, light yellow coloring with a white margin on the obverse, and dull yellow coloring on the reverse. On potato dextrose agar (Difco, Detroit, MI) at 30°C, a diffusible green pigment was observed. The maximum growth temperature was 50°C. Microscopic examination showed phialidic conidiogenous cells gradually narrowing at the end and producing columns of smooth cylindroidal conidia (3.6 to 4 μm by 1.3 to 1.9 μm) (Fig. 1B to D). The whole conidiophore, including the phialides, had rugose walls. Genomic DNA extraction, amplification, and sequencing of the ITS region were performed as previously described (1). For isolates 1 and 2, a BLAST search revealed 98% and 99% identity with recently published sequences of *G. argillacea* (7, 8), 96% and 98% with the *G. argillacea* type strain (AF033389), and 96% and 97% with *Talaromyces eburneus* (AB176614), respectively. Of note, the pairwise comparison between isolates 1 and 2 showed 98.3% of similarity. Antifungal susceptibility testing of both of the isolates was performed using a broth microdilution technique by following the guidelines for the testing of conidium-forming molds of the Antifungal Susceptibility Testing Subcommittee of the European Committee on Antibiotic Susceptibility Testing (AFST-EUCAST; 2008), with some modifications (6). Itraconazole (Janssen-Cilag, Issy-les-Moulineaux, France), voriconazole (Pfizer Central Research, Sandwich, United Kingdom), posaconazole (Schering-Plough Research Institute, Kenilworth, NJ), caspofungin (Merck and Co., Rahway, New Jersey), micafungin (Astellas Pharma, Osaka, Japan), and anidulafungin (Pfizer) were tested in RPMI 1640 medium supplemented with 2% glucose for all drugs, except for amphotericin B (Sigma-Aldrich), which was tested in AM3 medium (Difco, Becton-Dickinson), with a final inoculum size of 10^5 CFU/ml (35°C). Two reference strains, *Candida krusei* ATCC 6258 and *Candida parapsilosis* ATCC 22019, were included in each set of determinations to ensure quality control. The MICs of isolates 1 and 2, respectively, were the following: voriconazole (≥8 μg/ml), itraconazole (≥8 and 1 μg/ml), posaconazole (2 and 0.25 μg/ml), amphotericin B (8 and 4 μg/ml), caspofungin (0.25 and 0.5 μg/ml), micafungin (0.03 and 0.06 μg/ml), anidulafungin (0.03 μg/ml), and terbinafine (0.125 and 0.25 μg/ml).

The genus *Geosmithia* was proposed by Pitt (11a) to accommodate species formerly classified as *Penicillium* and having

unique, distinct characteristics, such as colony color (different from the gray-blue or gray-green colonies of *Penicillium* isolates), roughened conidiophores, acerose phialides, and cylindroidal conidia. *Geosmithia argillacea*, the anamorph of *Talaromyces eburneus*, is a thermophilic filamentous fungus that was first described in 1969 (13, 15). Although its habitat remains unknown, it has been recently shown to induce airway colonization in a limited number of patients with cystic fibrosis who had previously received itraconazole with or without voriconazole (2, 7). Of note, no association has been found between colonization detection and either age or lung function (2). No human case of invasive *G. argillacea* has been described yet. Invasive fungal infections occur in up to 43% of patients with chronic granulomatous disease (CGD) over time (3), mostly caused by *Aspergillus* spp. (11), and itraconazole has been shown to be beneficial as the primary prophylaxis of invasive aspergillosis in this setting (3). However, long-term azole prophylaxis during CGD has been associated with the emergence of multiple-resistant *Aspergillus fumigatus* or of new intrinsically less susceptible *Aspergillus* spp. (9, 14).

In the context of recent reports showing the emergence of infections due to other rare filamentous fungi in CGD patients (4, 14), we report here the emergence of *G. argillacea* as a cause of disseminated infection in two CGD patients receiving long-term azole prophylaxis. The clinical features of our two patients demonstrate that *G. argillacea* can be associated with subacute invasive presentation, consisting of at least pulmonary and contiguous bone involvement with presumed cerebral infections in both cases. We believe that recurrent isolation of this single fungus from sterile sites reinforces its pathogenic role, at least in CGD patients. Such a clinical presentation is similar to that of *A. nidulans* (5) or *Neosartorya udagawae* (14) infections in patients with CGD.

The only case of systemic infection due to *G. argillacea* has recently been reported in a German shepherd dog, an animal species known to be predisposed to disseminated aspergillosis (12). Of note, in this animal case, discospondylitis and sternal involvement were also described (8).

Importantly, the recently reported *Geosmithia* isolates (these two cases, cystic fibrosis cases, and the case reported in a dog) were initially misidentified as *Penicillium* spp. or *Paecilomyces* spp. (7, 8). In addition, a recent study showed that among 34 clinical isolates of *Paecilomyces variotii*, 4 were in fact *T. eburneus* (10). One case corresponded to colonization in a cystic fibrosis patient, and three others were isolated from blood or peritoneal dialysis fluid cultures (10). It is thus possible that *G. argillacea* might have been responsible for previous cases of systemic infections mistakenly reported as being due to *Penicillium* spp. or *P. variotii* also during CGD. We therefore recommend for all pathogenic molds identified at first as *Paecilomyces* or *Penicillium* that a careful microscopic observation be performed, looking for roughened conidiophores, cylindroidal conidia evocative of *Geosmithia* spp., and definite identification based on nucleotide sequencing.

The isolates found here had high MICs for itraconazole, voriconazole, and amphotericin B and variable ones for posaconazole. They exhibited low MICs for echinocandins and terbinafine, a result that is in accordance with recent data from the literature. Interestingly, our two patients received long-term azole therapy for prior episodes of *Aspergillus* infection. As one would expect, itraconazole and voriconazole were found inactive in 3/4 and 4/4 isolates of *T. eburneus*, respectively (10), and also were inactive against other emerging molds, such as *N. udagawae* in CGD patients (14).

One of the two patients recovered slowly while receiving a triple combination of antifungals, posaconazole, high-dose caspofungin, and terbinafine in combination with gamma interferon, but subsequently developed aseptic cerebral and undocumented medullary abscesses. The efficacy of these antifungals is concordant with the low MICs found against these antifungals. In conclusion, intrinsically multiply resistant *G. argillacea* isolates may emerge as a cause of disseminated infections in CGD patients receiving long-term azole therapy.

Nucleotide sequence accession numbers. The sequences for isolates 1 and 2 (481 and 489 bp, respectively) were deposited in GenBank under accession numbers HQ848389 and HQ599538, respectively.

We have no potential conflicts of interest.

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